treated smoked hake which was found to contain DMN in our previous studies[17]. A strong peak corresponding to DMNA (Fig. 1) thus confirms the identity of DMN.

Although we have only used the technique for the determination of DMN, it is anticipated that other volatile nitrosamines could be similarly converted to the nitramines and detected by GLC. It is hoped that the extreme sensitivity of the technique will be useful for studying the metabolism of various nitrosamines as well as for detecting their presence in the environment.

The author wishes to thank Mrs. BARBARA DONALDSON for technical assistance and Mr. R. C. O'BRIEN for taking the infrared spectrum.

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1 P. N. MAGEE AND J. M. BARNES, *Brit. J. Cancer*, 10 (1956) 114.
2 D. SCHMÄHL AND H. OSSWALD, *Experientia*, 23 (1967) 497.
3 J. SAKSHAUG, E. SÖGNEN, M. A. HANSEN AND N. KOPPANG, *Nature*, 206 (1965) 1261.
4 E. KRÖLLER, *Deut. Lebensm.-Rundschau*, 10 (1967) 303.
5 F. ENDER AND L. ČEH, *Food. Cosmet. Toxicol.*, 6 (1968) 569.
6 L. S. DU PLESSIS AND J. R. NUNN, *Nature*, 222 (1969) 1198.
7 R. PREUSSMANN, G. NEURATH, G. WULF-LORENTZEN, D. DAIBER AND H. HENGY, *Z. Anal. Chem.*, 202 (1964) 187.
8 W. J. SERFONTEIN AND P. HURTER, *Nature*, 209 (1966) 1238.
9 F. ENDER AND L. ČEH, *Alkylierend Wirkende Verbindungen*, Second Conference on Tobacco Research, Freiburg, 1967, p. 83.
10 H.-J. PETROWITZ, *Arzneimittel-Forsch.*, 18 (1968) 1486.
11 N. P. SEN, D. C. SMITH, L. SCHWINGHAMER AND J. J. MARLEAU, *J. Ass. Offic. Anal. Chem.*, 52 (1969) 47.
12 J. K. FOREMAN, J. F. PALFRAMAN AND E. A. WALKER, *Nature*, 225 (1970) 554.
13 J. H. HOWARD, T. FAZIO AND J. O. WATTS, *J. Ass. Offic. Anal. Chem.*, 53 (1970) 269.
14 O. G. DEVIK, *Acta Chem. Scand.*, 21 (1967) 2302.
15 J. H. ROBSON, *J. Am. Chem. Soc.*, 77 (1955) 107.
16 W. D. EMMONS AND A. F. FERRIS, *J. Am. Chem. Soc.*, 75 (1953) 4623.
17 N. P. SEN, D. C. SMITH, L. SCHWINGHAMER AND B. HOWSAM, *Can. Inst. Food. Technol. J.*, 3 (1970) 66.

Received May 4th, 1970

. Chromatog., 51 (1970) 301–304

:HROM. 4846

ieparation of carcinogenic nitrosamines on Sephadex LH-20

Chromatographic separations are well-established methods for the analysis of N-nitroso compounds. Thin-layer chromatography on silica gel or alumina[1] and a ariety of gas chromatographic methods have been successfully utilised for the sepaation and identification of these substances[2]. During our investigations of suitable ıethods for trace analysis of N-nitroso compounds[3–5] we examined whether gel hromatography could be employed as a separation technique for nitrosamines and ossibly also for the removal of interfering contaminants.

Materials and methods

All nitrosamines investigated were synthesised in our laboratory and purified by known methods; their purity was established by a gas chromatographic examination. Fifty percent aqueous methanol, analytical grade, was the solvent system of choice even for highly lipophilic N-nitroso compounds such as di-*n*-hexylnitrosamine.

A wheat flour concentrate was prepared by extraction of 1000 g of commercial wheat flour with dichloromethane in a Soxhlet for 12 h. The solvent was evaporated (rotatory) and the residue was partitioned in *n*-heptane–acetonitrile[3]. The acetonitrile phase was evaporated together with 100 g of alumina (neutral). The alumina with the adsorbed residue was filled in a column and eluted with 50% aqueous methanol; 50 ml were collected.

Sephadex LH-20 was allowed to swell for 12 h in the solvent and was then packed into a 1 × 100 cm column. The gel was washed with the solvent until the eluate showed no absorption in UV light at 230 nm. To prevent photolytic cleavage

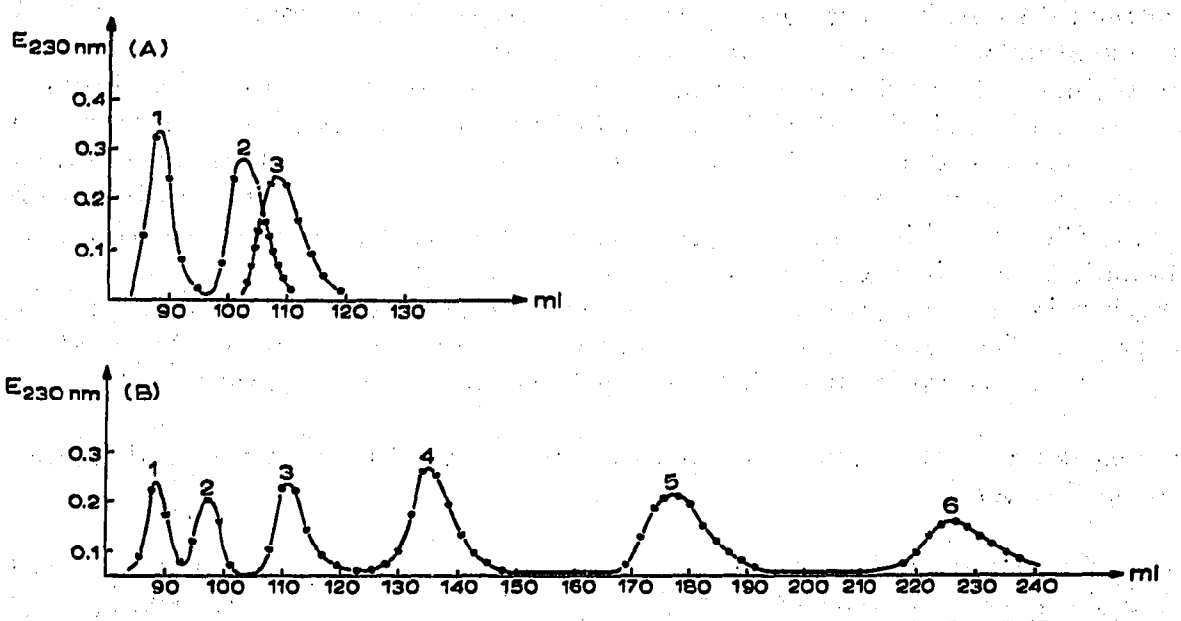

Fig. 1. Gel chromatography on Sephadex LH-20. Column, Sephadex LH-20, 100 × 100 mm; eluent, methanol–water (1 : 1), 4.3 ml/h; sample volume, 0.5 ml. (A) 1 = 19.4 μg methylethylnitrosamine; 2 = 26.4 μg methylbutylnitrosamine; 3 = 25.3 μg methylpentylnitrosamine. (B) 1 = 10.5 μg dimethylnitrosamine; 2 = 14 μg diethylnitrosamine; 3 = 21.5 μg di-*n*-propylnitrosamine; 4 = 50 μg di-*n*-butylnitrosamine; 5 = 74 μg di-*n*-pentylnitrosamine; 6 = 52 μg di-*n*-hexylnitrosamine.

of nitrosamines during chromatography, the column was screened from direct light with black paper. The compounds were applied in a sample volume of 0.5 ml, elution was performed at a rate of 4.3 ml/h and fractions of 2 or 3 ml were collected. Nitrosamine concentrations in the fractions were determined by UV spectroscopy at 230–235 nm, using a Zeiss DMR 21 recording spectrophotometer.

Results and discussion

As can be seen from Fig. 1, gel chromatography on Sephadex LH-20 resulted in an efficient separation of all six symmetrical di-*n*-alkylnitrosamines under the con-

ditions described above. Dimethylnitrosamine was eluted first, followed by diethylnitrosamine, di-*n*-propylnitrosamine and the higher homologues.

The elution sequence of the methylalkylnitrosamine homologues follows the same principle; thus methylethylnitrosamine is eluted before methylbutylnitrosamine which is followed by methylpentylnitrosamine. A similar separation as that shown in Fig. 1 was obtained with the application of 140–270 μg of symmetrical dialkylnitrosamines using a 2 × 100 cm column.

Whilst dialkylnitrosamine homologues which differ in two methylene groups were invariably well separated, the separation of nitrosamines with a difference of only one methylene group was incomplete (methylbutyl- from methylpentylnitrosamine).

The above results indicate that the separation is not based upon a gel filtration process. Apparently the elution behaviour of symmetrical and unsymmetrical nitrosamines under these conditions is determined primarily by absorption effects. The affinity of a given nitrosamine to the Sephadex LH-20 matrix increases with its lipophilic character and can be correlated with the distribution coefficient of the compounds in the system *n*-hexane–aqueous buffer (ref. 6, p. 115).

The usefulness of gel chromatography for the separation of micro-amounts of nitrosamines from biological materials was also investigated. A similar mixture of nitrosamines was dissolved in a 2-ml aliquot of the wheat flour concentrate and applied on the 2 × 100 cm column. Over the whole elution range in which nitrosamines were to be expected, UV-absorbing contaminants from the extract prevented the determination of the substances in the eluate by UV spectroscopy.

Although mixtures of pure nitrosamines can be successfully separated by gel chromatography on Sephadex LH-20, the method as described cannot be used for the separation of nitrosamines from interfering biological contaminants.

This investigation was carried out under contract with the International Agency for Research on Cancer.

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1 R. PREUSSMANN, G. NEURATH, G. WULF-LORENTZEN, D. DAIBER AND H. HENGY, *Z. Anal. Chem.*, 202 (1964) 187.
2 L. FISHBEIN AND H. FALK, *Chromatog. Rev.*, 11 (1969) 265.
3 G. EISENBRAND, P. MARQUARDT AND R. PREUSSMANN, *Z. Anal. Chem.*, 247 (1969) 54.
4 G. EISENBRAND, A. V. HODENBERG AND R. PREUSSMANN, *Z. Anal. Chem.*, 251 (1970) 22.
5 G. EISENBRAND, K. SPACZYNSKI AND R. PREUSSMANN, *J. Chromatog.*, 51 (1970) 503.
6 H. DRUCKREY, R. PREUSSMANN, S. IVANKOVIC AND D. SCHMÄHL, *Z. Krebsforsch.*, 69 (1967) 103.

Received May 26th, 1970